Moisture Analysis and Estimation of Crystalline α-Lactose in Whey Powders

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Abstract

Chemical titration, azeotropic distillation, and vacuum dehydration techniques were evaluated for determining moisture in spray-dried whey powders. Total moisture contents of cottage and Cheddar cheese whey powders are best determined with the Karl Fischer titration and toluene distillation methods, respectively. Free moisture, exclusive of water of hydration of α-lactose$\cdot H_2O$ crystals, may be measured by drying in vacuo at 65 C at 100 torr for 6 h. Vacuum dehydration at higher temperature and prolonged toluene distillation of cottage cheese whey powder are complicated by lactic acid volatilization and by powder decomposition. High vacuum drying of sweet whey powder releases some of the water of hydration of α-lactose. Crystalline lactose contents of whey powders can be estimated within 10% of polarimetrically determined values by taking the differences between total and free moisture values. Thermogravimetry can provide a similar estimate of lactose crystallinity in dried products other than cottage cheese whey powder.

Introduction

The expanding commercial development of dried whey products, particularly cottage cheese whey, requires a rapid and accurate method for moisture determination. Such analysis is not only needed for product standardization, relevant to pricing, but also because certain physical properties of the powders, including resistance to caking, are moisture sensitive. It is necessary to know the amount of water and its mode of attachment to the powder, e.g., as the water of hydration of α-lactose$\cdot H_2O$ or as adsorbed water.

Procedures with dried milks include toluene distillation (1), drying in a vacuum oven (3), or Karl Fischer titration (12). Comparative studies of these methods have been reported (6, 8, 10). Della Monica and Holden (6) found toluene distillation and Karl Fischer titration methods agree for determinations of moisture in dried whole milk; however, the Karl Fischer technique was less precise above 7% moisture. De Moor and Hendricx (8) reported that toluene and Karl Fischer methods determine the total water content of milk and whey powder, but the vacuum drying method determines none or only part of the α-lactose hydrate water. They also stated that only Karl Fischer and vacuum drying methods gave satisfactory results with roller-dried whey.

Lactose hydrate water may be computed from the crystalline α-lactose content as determined polarimetrically (14); however, such analyses cannot be completed in the laboratory in a single day. Thermogravimetric analysis (TGA) can detect the water of hydration in α-lactose (4) and skim milk powders (5); however, further study is needed to establish the reliability of thermogravimetry for determining the water of hydration of lactose in whey powders. Similarly, variations in time, temperature, and pressure in vacuum oven dehydration might be considered for distinguishing between sorbed water and crystal hydrate water. Drying or distillation methods, however, are subject to the interference of volatile components other than water which may be in the powder or formed during analysis (2).

In the present investigation we compared techniques for moisture determination. The purposes of this work were to select the best method for determining both total moisture and water of hydration in dried whey and to determine whether lactic acid volatilizes and interferes with water analyses. A further objective was to assess the feasibility of using water of hydration data to obtain a quick estimate of the crystalline lactose content of a

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whey powder without actually measuring the lactose content of the powder. Though only approximate, such estimation would be advantageous, as conventional procedures for measuring crystalline lactose are time-consuming.

Materials and Methods[3]

Cheddar and cottage cheese whey powders were obtained from commercial sources or spray-dried in the Dairy Products Laboratory Pilot Plant. Acid whey was prepared for comparison by precipitation of casein from skim milk with HCl, and the resulting whey was freeze-dried.

The moisture analysis methods compared in this study included toluene distillation, Karl Fischer titration, and vacuum dehydration.

Toluene distillation. The accepted apparatus (1) employing a 5-ml Bidwell-Sterling distilling receiver was used. Routinely, sufficient powder to yield 1 to 2 ml H_2O was weighed into a 300-ml Erlenmeyer flask, 100 to 130 ml reagent grade toluene were added, and the distillation was for 75 min as described (1) for milk powder. This distillation procedure was tested for completeness by continuing the distillation for 6 h with samples of whey powders and with skim milk powder for comparison. Distilled water collected during analysis was examined for lactic acid since it would detract from the accuracy of the analysis.

Karl Fischer. A Beckman KF-3 Aquameter equipped with duo platinum electrode was used for all titrations. The direct titration technique used Karl Fischer Reagent stabilized, single solution (Fischer Scientific Co.) as the titrant. The procedure was similar to that adopted for whole milk powder analysis by Della Moni and Holden (6). Exactly 50 ml dry CH_3O were added to a 125-ml flask containing 1 2 g whey powder, and the mixture w agitated for 1 h with a magnetic stirrer to e tract the H_2O from the powder. After allo ing the extraction mixture to settle for 15 m a 10 ml aliquot of the methanol extract co taining 10 to 20 mg H_2O was transferred to t 300-ml titration vessel of the Aquameter. D ing sample transfer the system was purg with dry nitrogen to minimize moisture sorption from the atmosphere. The titrant quired to pre-dry the methanol was det mined daily and constituted a blank value be subtracted from all standardization analysis titrations. The water equivalent of Karl Fischer reagent was determined w either pure α-lactose monohydrate or a so tion containing 150 to 200 mg H_2O/100 CH_3OH as standards. The H_2O-CH_3OH s tions were prepared daily.

Vacuum dehydration. A method similar that of Anselmi and Cesari (2) for milk p der was used. Samples weighing 1 to 2 g v placed in glass weighing bottles and dried 16 h in a vacuum oven at 100 torr and 6 This method was adopted after establis that no lactic acid is removed from the v powders under these conditions. Studies also to establish minimum drying time and propriate pressure for determining the called free or nonhydrate water conten whey powders.

The feasibility of using thermogravim analysis as an alternate method for deter ing hydrate water in whey powders was e ated. Analyses were as described (4, 5) a Cahn RG recording electrobalance an Little Gem TGA accessory of the Cahn I ment Company.

Whey powders were analyzed for cryst α-lactose polarimetrically according to the

[3] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. Precision of analytical methods for moisture.

Method	Cottage cheese whey powder			Cheddar cheese whey powd		
	No. samples analyzed	Avg deviations from the mean (range)	Mean value of the avg deviations	No. samples analyzed	Avg deviations from the mean (range)	Mean of th devi
Karl Fischer titration	28	.01-.18	.08	23	.03-.61	
Toluene distillation	9	0-.44	.18	13	0-.50	
Vacuum oven (65 C, 100 torr, 16 h)	19	0-.06	.03	12	0-.05	

cedure of Sharp and Doob (14). Optical rotation after dissolution of the powder was read with a Perkin-Elmer Model 141 automatic polarimeter with digital readout.

Lactic acid was determined by the procedure of Lawrence (11).

Results and Discussion

A total of 28 cottage and 23 Cheddar cheese whey powders were examined. The number analyzed by each method is shown in Table 1. Most of the Karl Fischer analyses and all of the toluene and vacuum oven analyses were performed in duplicate. In addition seven Cheddar and six cottage cheese wheys were analyzed by the Karl Fischer method in triplicate to further gauge the reproducibility of these analyses. The precision of the analytical methods was assessed by determining the average deviation from the mean for the analysis of each powder and the mean value of the average deviations for each analytical method (Table 1). Standard deviations reported by De Moor and Hendricx (9) indicate somewhat more precision in their analysis of whey powder. Della Monica and Holden reported confidence limits ($P=.05$) of $\pm.130$ to $\pm.292$ for Karl Fischer and $\pm.130$ to $\pm.199$ for toluene analyses of moisture in whole milk powder (6).

Representative moisture data are in Table 2 to demonstrate differences in values with each of the methods. Only six samples of each type of whey powder are included in this table for the sake of clarity and brevity. All analyses of each of these powders were simultaneous to insure uniform moisture contents and avoid interactions with the atmosphere.

Toluene distillation yielded higher moisture values than Karl Fischer titration in most cases. These differences were almost entirely within experimental error for the cottage cheese whey powders but were more pronounced with the Cheddar cheese whey powders.

In nearly all cases vacuum dehydration at 65 C yielded lower moisture values, probably because the water of hydration of the crystalline α-lactose monohydrate is not vaporized under these conditions (8). These data suggest that the vacuum method determined only the free water (water not held in the α-lactose crystal lattice) while the Karl Fischer or toluene techniques determine the total water content.

The toluene distillation values were obtained by the standard procedure for milk powder (1) wherein distillation is continued for 75 min. When distillation was continued longer to test the procedure for completeness, results in Fig. 1 were obtained. The volumes of water distilled from the Cheddar cheese whey powder and the skim milk control powder remained constant after 75 min; however,

TABLE 2. Moisture and crystalline lactose contents of whey powders.

Powder	Moisture (% wet weight)			Hydrate water (% wet weight)		Crystalline lactose (% total lactose)	
	Karl Fischer titration	Toluene distillation	Vacuum oven 100 torr 65 C 16 h	Calculated	Estimated	Polarimetric value	Estimated from hydrate H_2O
Cottage cheese whey							
1	8.9	9.4	6.1	2.8	2.8	91.9	87.8
2	5.7	5.9	3.5	1.9	2.2	60.5	68.7
3	6.1	6.1	4.0	1.9	2.1	59.5	65.8
4	2.9	3.2	.7	2.2	2.2	61.9	68.7
5	3.4	3.0	1.4	1.9	2.0	56.3	62.7
6	3.3	3.9	2.8	.4	.5	12.0	15.7
Cheddar cheese whey							
1	5.2	5.1	2.5	2.8	2.6	83.3	73.7
2	3.0	4.1	2.1	2.5	2.0	69.2	56.7
3	2.8	3.8	2.0	1.7	1.8	49.9	51.0
4	2.7	2.7	2.4	.2	.3	6.7	8.5
5	2.1	3.6	1.2	2.5	2.4	68.9	68.0
6	2.7	3.4	.9	2.5	2.5	69.3	70.9

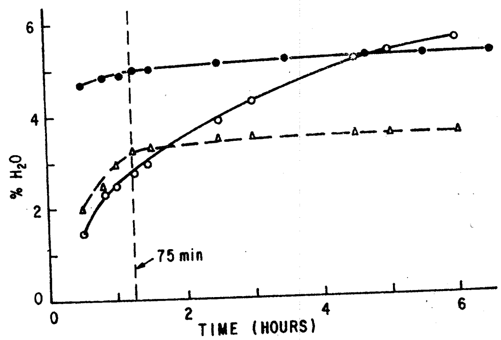

FIG. 1. Influence of length of toluene distillation period on the moisture analysis of (△) Cheddar and (○) cottage cheese whey and (●) skim milk powders.

water continued to be distilled from the cottage cheese whey powder for the entire 6 h that the experiment was continued. These data suggest that the toluene distillation method is of questionable value for the moisture analysis of cottage cheese whey powder though there is agreement between values at the standard distillation period and those from the Karl Fischer titration.

The distillate collected after 75 min represents either volatile constituents other than water formed through the decomposition of the whey solids. Analysis of distillates (Table 3) showed a substantial increase in the lactic acid content of the aqueous phase of the distillate from cottage cheese whey powder after 6 h continuous distillation. The measured quantity of lactic acid is, however, insufficient to account for the entire excess volume of distillate collected between 75 min and 6 h. It is conceivable that during distillation the cottage cheese whey solids are subject to a decomposition reaction yielding water as one of the products. The equilibrium constant for this reaction may be such that the reaction will be inhibited by any water in the system. Thus, the water distilled during the first 75 min may actually represent the moisture initially present in the

TABLE 3. Lactic acid in aqueous phase of toluene distillates from whey powders.

Powder	Lactic acid (mg)	Water (ml)	Time (h)
Cottage cheese whey	7.8	.70	1.25
Cottage cheese whey	43.7	1.38	6.0
Cheddar cheese whey	.8	.81	1.25
Cheddar cheese whey	3.8	.88	6.0

powder while that collected after 75 min represents water formed through decomposition of the whey solids.

The differences between some of the Karl Fischer and toluene analyses of the sweet whey powders are more difficult to understand. It is possible that some of the spray-dried powder particles may be so structured as to impede the methanol extraction of water. The Karl Fischer values in Table 2 were all determined by titrating after an extraction period of 1 h. When samples of Cheddar whey powder no. 5 were extracted with CH_3OH for periods of 1, 2, 2.5, 4, 5, and 6 h, values of 2.09, 2.39, 2.41, 2.88, 2.91, and 3.09% H_2O were obtained thus demonstrating a time dependence in the extraction procedure. This behavior is unusual, as Della Monica and Holden (6) reported no difficulties in moisture extraction from vacuum foam-dried whole milk powder and De Moor et al. (7) reported that variations in contact time with methanol (5 to 60 min) had no influence on the moisture content of milk powder.

The (with difficulty) extractable water cannot be associated with an inability to extract the water of hydration of α-lactose$\cdot H_2O$ from within the crystal lattice, as titrations with pure α-lactose$\cdot H_2O$ powder yielded the theoretical value of 5% H_2O consistently. Zimmerman (17) reported that water contents of maltose and lactose monohydrates could be determined by Karl Fischer titration after a preliminary extraction period of .5 to 1 h if the materials were finely ground.

These results (Table 2) suggest that toluene distillation be considered the method of choice for analysis of total moisture content of Cheddar cheese whey powder. On the other hand, we favor the Karl Fischer titration method for total moisture analysis of cottage cheese whey powder because there will always be uncertainty in deciding when to stop toluene distillation. Furthermore, the mean deviations (Table 1) indicate greater precision in the Karl Fischer analysis of the cottage cheese whey powder.

Data in Table 4 show that varying the pressure between 25 and 100 torr and extending the evacuation period from 6 to 16 h had negligible effects on the vacuum oven (65 C) determined moisture values of cottage cheese whey except the small differences with powders no. 4 and 5. Conversely, lowering the pressure to 25 torr and extending the evacuation period to 16 h increased the water evolution from the Cheddar cheese whey powders

TABLE 4. Effect of pressure and evacuation time on whey powder moisture values determined by vacuum drying.

Powder	Moisture (% wet weight)			
	25 torr		100 torr	
	6 h	16 h	6 h	16 h
Cottage cheese whey				
1	3.80	3.85	4.00	3.95
2	3.21	3.36	3.36	3.37
3	4.34	4.30	4.35	4.42
4	2.72	3.01	2.54	2.45
5	3.00	3.15	2.81	2.76
Cheddar cheese whey				
1	2.59	3.48	2.11	1.92
2	3.11	3.44	2.50	2.49
3	2.52	2.92	2.16	2.14
4	2.44	2.80	2.10	1.96
5	5.29	5.12	5.01	4.93

except powder no. 5. The sweet whey data may be understood by assuming that the water of hydration of α-lactose cannot be removed at 65 C under a pressure 100 torr; however, some of the hydrate water may be removed when the pressure in the oven is reduced to 25 torr. Sweet whey powder no. 5 shows little additional water removal after pumping for 16 h at 25 torr as this powder contained only 6.7% crystalline lactose. Control experiments with pure crystalline α-lactose $\cdot H_2O$ confirmed this explanation. Indeed, De Moor and Hendricx (8) reported that part of the hydrate water may be removed in the vacuum oven at 75 C and under a pressure of 60 torr.

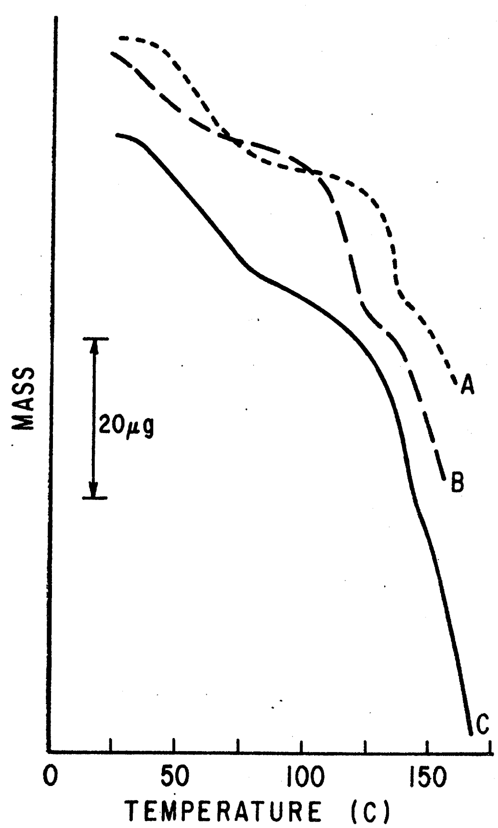

FIG. 2. Thermogravimetry curves for: A, 5.032 mg spray-dried Cheddar cheese whey powder; B, 5.004 mg lyophilized HCl whey; and C, 4.608 mg spray-dried cottage cheese whey powder.

Results of thermogravimetric analysis of cottage and Cheddar cheese whey powders and a control sample of lyophilized acid whey from HCl-precipitated casein are shown graphically in the thermograms of Fig. 2. The Cheddar and acid whey powders display three distinct steps of mass loss with increasing temperature corresponding to: (1) desorption of free or sorbed water, (2) evolution of water of hydration from the α-lactose crystals, and (3) thermal decomposition of the dried material. The TGA curve for cottage cheese whey powder, however, can only be separated into two steps corresponding to the desorption of the so-called free water at low temperature followed by evolution of crystal hydrate water concomitant with decomposition of the powder. Apparently, some factor related to the composition of cottage cheese whey inhibits removal of the water of hydration of α-lactose until reaching a high enough temperature to decompose the powder. The cottage cheese whey thermogram is complicated further in that deposition of lactic acid was noted on the cold portion of the balance hangdown tube above the TGA furnace when the powder was at a temperature high enough for evolution of the water of hydration. These results are compatible with the vacuum oven data which showed neither the removal of crystal hydrate water nor evolution of lactic acid from cottage cheese whey powder when held at 65 C.

The significance of lactose crystallinity to the processing and quality of dehydrated dairy products is widely recognized (13, 16); however, satisfactory methods for the rapid direct determination of lactose in the crystalline form have not been available. The α-lactose content determined polarimetrically (14) after dissolution of the powder is often taken as an indicator of the degree of crystallinity. The method is time-consuming and yet is only an approximation. Susi and Ard (15) have recently reported on a far infrared spectroscopic method for α-D-lactose crystallinity performed with the

TABLE 5. Crystalline lactose content of sweet whey powders.

Sample	Thermogravimetric estimate (% of lactose)	Polarimetric value (% of lactose)
1	22.7	24.7
2	45.4	39.6
3	45.4	40.7
4	66.9	69.6
5	76.9	91.7
6	80.3	91.8
7	35.2	34.9
8	46.8	49.4
9	83.7	69.6
10	92.5	95.0

solids directly and requiring only 1 h. They reported agreement within 5% between the spectroscopic and polarimetric methods on whey solids.

The multistep thermograms (Fig. 2) for sweet whey powder suggest using thermogravimetry to measure the water of hydration of the crystalline α-lactose in whey powder to provide a rapid method for determining the crystalline α-lactose content of the powder. Estimated values of crystalline lactose content of several sweet whey powders, determined by TGA, are compared with polarimetric data in Table 5. The estimates were arrived at by computing the hydrate water content from the appropriate segment of the TGA curves and assuming a total lactose content of 67%. This assumed value was based on our general experience with sweet whey powders in the laboratory and is the average lactose content of more than 15 different Cheddar cheese whey powders. It is possible to estimate the crystalline α-lactose content of sweet whey powder within 10% of the polarimetric value in approximately 30 to 45 min without knowing the actual lactose content of the powder. The major difficulty in the TGA method is in accurately establishing points on the TGA curve when the evolution of crystal hydrate water begins and ends. Furthermore, thermogravimetry cannot be used for this purpose with cottage cheese whey powder.

The α-lactose$\cdot H_2O$ content may also be estimated by taking the difference between the total and so-called free moisture contents of the [illegible] tillation (Cheddar cheese whey powder) values are used for total moisture. Estimates were based on assumed values of 67.0% lactose in Cheddar cheese whey powder and 60.5% lactose in cottage cheese whey powder, the average value of 15 different powders analyzed in our laboratory. The estimated values in Table 2 were calculated from oven data after evacuating for 16 h; however, the data in Table 4 show that an equally good estimate can be obtained after drying for only 6 h. It is thus possible to obtain a reasonably accurate (within 10%) estimate of the crystalline lactose content of a whey powder by this method within a single laboratory day. This approach should prove useful in dairy manufacturing installations where only limited laboratory facilities are available. Furthermore, expensive equipment such as polarimeters or infrared spectrophotometers is not required.

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